THE JEREMIAH METZGER LECTURE

AN ANALYSIS OF CIRCADIAN RHYTHMS IN HUMAN ADRENOCORTICAL SECRETORY ACTIVITY

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Rhythmicity has long been known to be a characteristic of many biological functions, and the mechanisms underlying biological rhythms have been of increasing interest to investigators during recent years. Some biological rhythms have been referred to as "endogenous" (self-sustained) since they are not closely correlated with any obviously rhythmic phenomena in the external environment. Others, which are obviously correlated with cyclic changes in the external environment, have been called "exogenous" (forced). Halberg[1] has used the term "circadian" ("circa"—about, "dies"—day) in referring to biological rhythms having a period of about 1 day.

Several studies have provided evidence that adrenal steroids are secreted on a cyclic schedule with a period of about 24 hours.[2-6] By rescheduling the activities of normal subjects, it has been possible to change the hour at which adrenal steroid production rises and falls.[7] All studies prior to the present one, however, have been alike in showing the cycle to occur only once a day. Thus, the idea has gained currency that the mechanism underlying the adrenal cycle has inherent circadian periodicity.[8] The present paper offers evidence that the one day:one cycle pattern of adrenal activity is by no means immutable.

The term, "adrenal cycle", represents an oversimplification which must be abandoned at this point in the discussion; for the two major adrenal hormones, cortisol and aldosterone, have different sources, different functions, and different regulatory mechanisms. It is necessary, therefore, to consider the apparent circadian rhythms of these two adrenal steroids separately.

Mechanisms Underlying the Apparent Circadian Rhythm in Aldosterone Secretion

In normal, active people the circadian cycle for aldosterone secretion as well as that for cortisol secretion is characterized by a "crest"

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early in the day and a "trough" late in the day. This suggested to Bartter *et al.*[9] that both the aldosterone and the cortisol cycles might be secondary to the cyclic secretion of adrenocorticotropic hormone (ACTH). However, since normal subjects are upright during the day and recumbent at night, it would be equally reasonable to suppose that posture controls aldosterone secretion; and, indeed, experimental evidence supporting this view was published several years ago by Muller *et al.*[10]

To assess the relative importance of ACTH and of posture in determining the circadian rhythm of aldosterone secretion, two types of experiments have recently been performed in our laboratory by Wolfe *et al.*[11] In one type of experiment, the circadian rhythmicity in ACTH secretion was eliminated by the continuous suppression of this hormone with dexamethasone, administered orally in doses of 0.5 mg. every 6 hours. The continuous suppression of ACTH reduced cortisol secretion, as reflected by 17α-21-dihydroxy-20-keto-corticosteroid (17-OHCS) excretion, to negligible values at all times of the day, but the circadian rhythm in aldosterone secretion persisted. In the second type of experiment, ACTH was not suppressed; but posture was held constant by having normal subjects remain recumbent throughout the 24-hour day. Under these conditions, the circadian rhythm in aldosterone secretion vanished, but the circadian rhythm in 17-OHCS excretion persisted. It appears, therefore, that posture is more important than ACTH in determining the usual circadian rhythm in aldosterone secretion.

Finally, 3 experiments were performed in which the postural stimulus to aldosterone secretion was deliberately set in opposition to the usual circadian cycle. Three normal subjects were kept recumbent for three days, during which time a circadian rhythm in aldosterone secretion could not be discerned. They were then allowed to be upright from 8 p.m. to 8 a.m., the time when aldosterone secretion is usually at a minimum. In each experiment, the assumption of upright posture was accompanied by an increase in aldosterone secretion. After the subjects resumed the recumbent position at 8 a.m., their aldosterone secretion decreased to the low levels previously observed during recumbency. Thus, reversal of the daily schedule of postural changes resulted in reversal of the circadian rhythm in aldosterone secretion (Fig. 1).

Recent studies have been carried out to elucidate the mechanism through which the assumption of an upright posture leads to an increase in aldosterone secretion. Upright posture results in pooling of blood in the dependent parts of the body and a decrease in blood volume.[12, 13] There is reason to believe that decreases in effective blood volume stimulate the production of renin by the kidneys.[14] Renin cata-

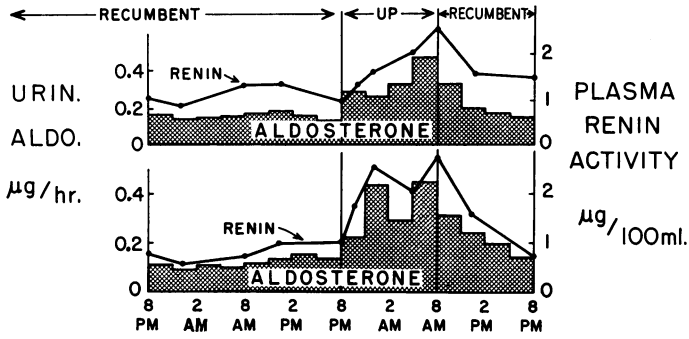

FIG. 1. Urinary aldosterone and plasma renin activity of two normal subjects on regimens of constant food and water intake (every 3 hours) and controlled activity. Dietary intake of sodium was 10 mEq. per day for the subject represented in the upper graph and 30 mEq. per day for the subject represented in the lower graph.

lyzes the formation of angiotensin,[15, 16] and angiotensin has been shown to be an effective stimulus to adrenocortical secretion of aldosterone.[17-19] It was considered probable, therefore, that the effects of posture on aldosterone secretion were mediated by changes in renin production. In order to assess the plausibility of this line of reasoning, plasma renin activity was assayed in parallel with aldosterone in several experiments carried out in normal subjects. Plasma renin activity rose upon the assumption of upright posture and fell upon resumption of a recumbent position. It appears, therefore, that the usual circadian rhythm in aldosterone secretion is not necessarily related to any rhythmic phenomenon of the external environment, nor is it governed by an inborn biological clock with a period of approximately 24-hours. It appears, rather, that aldosterone secretion rises during the early part of the day principally because plasma renin activity rises and that plasma renin activity rises during the early part of the day because that is when people get out of bed.

It must be added, however, that the physiological adjustment to postural change is complex. The increases in renin and aldosterone which occur in response to upright posture are of limited duration. After normal subjects have been upright for several hours, secondary decreases in plasma renin activity and aldosterone occur despite continued maintenance of the upright posture. Studies are now in progress to determine the basis for these secondary adjustments to posture.

Circadian Rhythm in Sodium Excretion

A circadian rhythm in sodium excretion has been observed repeatedly.[20-23] Inasmuch as adrenal steroids are important regulators of sodium excretion, the question has been asked whether the circadian

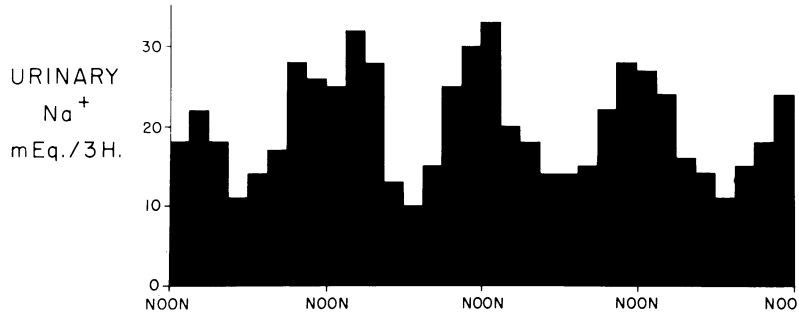

FIG. 2. Urinary sodium of a patient who had previously undergone bilateral adrenalectomy and who, during this study, was maintained on a 3 hourly regimen of constant diet, 5 mg. of cortisol, and 10 μg of fludrocortisone. She was recumbent at all times except for 15 minutes at the beginning of each 3 hour metabolic period when she was sitting up or walking about.

rhythm in sodium excretion could be a function of a circadian rhythm in adrenal function. Most investigators have concluded that the sodium rhythm is not secondary to the steroid rhythm because the maximum rate of sodium excretion coincides roughly with the maximum in steroid secretion, which is in contrast to what would be expected if the excretion of sodium were controlled principally by the adrenals.[9]

Doe *et al.*[21] have shown that the circadian rhythm in sodium excretion persists when steroidal and dietary factors are held constant throughout the day. Recently we have confirmed Doe's observations while holding constant yet another determinant of sodium excretion, *viz.*, posture. These studies were performed in 3 patients with Addison's disease who were maintained for several days on cortisol, 5 mg. every 3 hours; fludrocortisone, 10 μg. every 3 hours; and identical dietary and fluid intake every 3 hours. The patients were out of bed for 15 minutes at the beginning of each 3 hour period but were recumbent at all other times. Urine was collected every 3 hours for measurements of creatinine and electrolyte excretion. Despite the rigid control of all of these variables, a definite circadian rhythm in sodium excretion was present with lower values occurring during the night than during the day (Fig. 2). From these observations we may conclude that there is some, as yet unknown, mechanism which confers circadian rhythmicity on the renal excretion of sodium.

Mechanisms Underlying the Apparent Circadian Rhythm in Cortisol Secretion

Many studies have shown that there is a circadian rhythm in cortisol secretion that is reflected both in the plasma concentration[4-6] and urinary

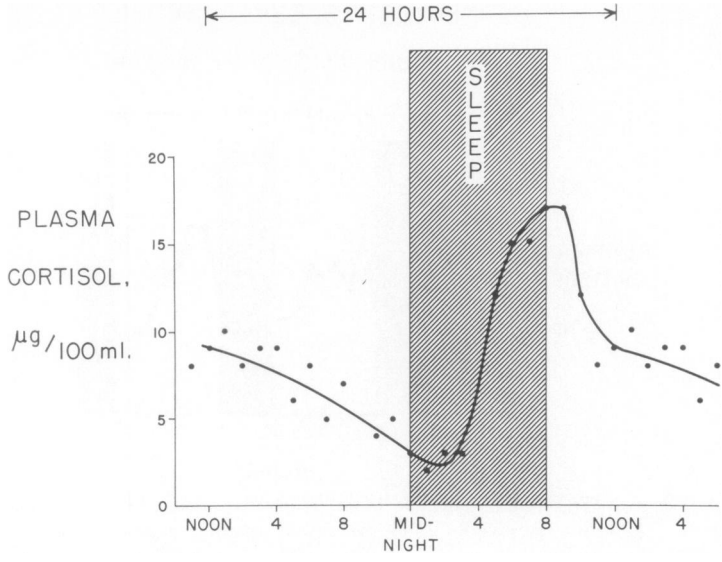

FIG. 3. Plasma 17-OHCS ("cortisol") of 5 normal subjects who were asleep from midnight until 8 a.m. and awake at all other times. Each point represents the mean of 5 subjects. Blood was sampled using indwelling venous catheters so that sleep would not be disturbed.

excretion of 17-OHCS.[3-5] In normal subjects on an ordinary schedule of daytime activity and nocturnal sleep, plasma 17-OHCS "crest" at about 8 a.m.; then gradually decline to a "trough" between 8 p.m. and midnight. At approximately 4 a.m., the plasma 17-OHCS begin to rise, again reaching a maximum at about 8 a.m. (Fig. 3).

Three lines of evidence support the view that the rhythmic rise and fall of plasma 17-OHCS are secondary to rhythmic change in plasma ACTH. *First*, in normal subjects the plasma concentrations of ACTH rise and fall at the same time of day as plasma 17-OHCS.[24] *Second*, addisonian patients maintained on constant infusions of cortisol do not have a discernible circadian rhythm in plasma 17-OHCS. Therefore, the plasma 17-OHCS rhythm does not appear to be secondary to a circadian rhythm in the rate of metabolic degradation of cortisol. *Third*, by administering ACTH at a constant rate to normal subjects over a 24-hour period, it is possible to abolish the circadian rhythm in plasma ACTH and thereby abolish the circadian rhythm in plasma 17-OHCS.[24]

A number of investigators have attempted to define the mechanism underlying the ACTH-cortisol rhythm. Katz[25] has shown that it is not dependent upon postural changes, since it persists in people kept at constant bed rest. Migeon *et al.*[4] have shown that it is not governed by visual

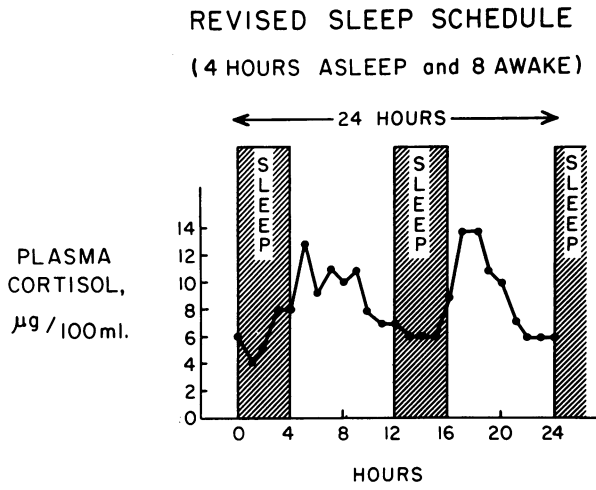

FIG. 4. Plasma 17-OHCS ("cortisol") of 6 normal subjects who had become adapted to a 12-hour sleep-wake schedule. Each point represents the mean of 6 subjects. Blood was sampled using indwelling venous catheters.

phenomena, since it persists in totally blind people. Perkoff *et al.*[7] have shown that a 12 hour shift in the sleep-wake schedule results in a corresponding 12 hour shift in the plasma 17-OHCS cycle. It appears, therefore, that the ACTH-cortisol rhythm is somehow governed by sleep-wake activity and not directly by a periodic change in the external environment. The concept that the ACTH-cortisol cycle is based on an inborn mechanism having circadian periodicity has been widely accepted. Before one can be satisfied with the conclusion that the ACTH-cortisol rhythm is *inherently circadian* (one day:one cycle), however, it would seem necessary to see what would happen if the sleep-wake cycle were to be rescheduled with a noncircadian period.

In the series of experiments illustrated in Figure 4, nine normal subjects adhered to a schedule which required them to be asleep 4 hours, then awake 8 hours, in each 12 hour period. Plasma for 17-OHCS determination was sampled through indwelling venous catheters every hour. On this schedule, which provided two sleep-wake cycles every 24 hours, six of these subjects rapidly developed plasma cortisol rhythms characterized *not by one but by two cycles every 24 hours.* Plasma 17-OHCS were highest soon after the subjects awakened and fell to low values about 3 or 4 hours later. The ease with which the plasma 17-OHCS were shifted from one to two cycles per day would suggest that the cyclic regulation of ACTH-cortisol secretion is dependent upon the sleep-wake cycle rather than some inborn mechanism with immutable circadian periodicity.

ACTH-Cortisol Rhythms in Various Disease States

Cushing's Syndrome. The chemical common denominator of Cushing's syndrome is excessive production of cortisol. Three causes of Cushing's syndrome are recognized; *first*, autonomous production of cortisol by an adrenal neoplasm; *second*, excessive production of cortisol by the adrenal glands in response to "ectopic" ACTH produced by tumors of various organs; and, *third*, excessive production of cortisol by the adrenal glands in response to secretion of ACTH by the pituitary.[26] Regardless of the cause of the cortisol excess, most patients with Cushing's syndrome fail to show a normal circadian rhythm in plasma 17-OHCS; in particular, the normal decrease in 17-OHCS fails to occur late in the day. This abnormality is frequently of value in establishing a diagnosis of Cushing's syndrome.[21, 27, 28]

Addison's Disease. Adrenocortical insufficiency serves as a stimulus to ACTH secretion. Teleologically, this may be regarded as a compensatory effort on the part of the pituitary to correct the cortisol deficiency. The question has been raised whether, in the presence of a steady deficiency of cortisol, the compensatory response of the pituitary would lead to a continuously high rate of secretion of ACTH and thereby abolish the circadian rhythm in plasma ACTH levels. Experiments designed to answer this question have recently been published from this department.[29] It was found that when addisonian patients were maintained on 12 small, equal doses of cortisol given every 2 hours, their plasma 17-OHCS levels were constantly subnormal and their ACTH levels became elevated. Despite the fact that plasma 17-OHCS were subnormal at all times, these patients, nevertheless, exhibited prominent circadian rhythms in plasma ACTH concentrations. It would appear that the influence of sleep-wake activity on ACTH secretion must be very powerful if it can bring about a decrease in the secretion of this hormone late in the day even in the presence of continuous cortisol deficiency.

Recovery from Prolonged Pituitary-Adrenal Suppression

Although it may be of no importance to the addisonian patient whether he secretes ACTH all of the time or only a few hours each day, these observations might have important implications for another group of patients with adrenal insufficiency, i.e., those recovering from prolonged pituitary-adrenal suppression. Recent studies[30] have shown that the recovery of normal pituitary-adrenal function following correction of iatrogenic Cushing's syndrome or Cushing's syndrome due to adrenocortical tumor is characterized by three phases. There is an

initial phase in which both plasma cortisol and plasma ACTH concentrations are subnormal, an intermediate phase in which plasma cortisol is subnormal despite the fact that plasma ACTH (at 6 a.m.) is supernormal, and a final phase in which cortisol and ACTH levels both return to normal. The entire process of recovery often requires several months. The slowness of recovery presents a teleological paradox; for, during the intermediate phase, the pituitary is capable of secreting large quantities of ACTH (which it does in the morning) but, nevertheless, fails to secrete ACTH for a sufficient period each day to bring daily cortisol production to normal. If, in the presence of cortisol deficiency, the pituitary gland were to maintain plasma ACTH at the 6 a.m. level throughout the day, adrenocortical function would be restored to normal far more rapidly than is actually the case. This failure of the pituitary to respond to cortisol deficiency with a *continuous* compensatory elevation of ACTH secretion is both inconvenient for the patient and puzzling to the physiologist.

Summary

The major rhythms in adrenal steroid secretion appear to be related to habitual patterns of activity and are not related in any fixed way to the external environment. They exhibit distinct circadian (approximately 24 hour) periodicity only if habitual activity patterns follow a circadian schedule. The dominant factor conferring apparent circadian rhythmicity upon aldosterone secretion is the effect of upright posture which acts apparently by stimulating the renal production of renin. The dominant factor conferring apparent circadian rhythmicity upon ACTH (and thereby cortisol) secretion is the sleep-wake schedule. By rescheduling postural and sleep-wake patterns, it is possible to abolish the 24-hour periodicity of adrenal steroid secretion. The concept that there is an adrenal cycle with inherent periodicity of about 24 hours cannot be accepted without further reappraisal.

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